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# Gliadin antibody detection in gluten enteropathy

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## SUMMARY

*Circulating antigliadin antibody has been described in patients with gluten enteropathy although the prevalence varies in different studies. It has been suggested that the investigation for antigliadin antibody might be useful as a screening test. The object of the present study was to evaluate two different techniques for assaying these antibodies — an indirect immunofluorescent method and an enzyme-linked immunosorbent assay (ELISA). Antibodies were assayed in the sera of 102 patients in whom jejunal biopsies were also obtained. The specificity of both tests was greater than 95%, and the correlation between the presence of antibody and histology was significant ( $p < 0.005$ ), though the sensitivity of each test was less than 70%.*

## INTRODUCTION

Detection in patients with gluten-sensitive enteropathy of circulating antibody to gluten or to one of its components, gliadin, might obviate the need for jejunal biopsy in diagnosis. However, the prevalence and specificity of tests for antibody have varied in different studies. In part, this may be due to differences between adults and children,<sup>1</sup> the use of different laboratory techniques,<sup>2,3</sup> and the assessment of different fractions of anti-gliadin antibodies. The IgA fraction has been shown to be more specific than IgG or IgM<sup>4,5,6</sup> and, more recently, antibody to  $\alpha$  gliadin was described as being more sensitive and specific for coeliac disease than antibody to other wheat proteins tested.<sup>7,8</sup> So far, no assay has yet proved to be an entirely satisfactory screening test for gluten enteropathy.

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The purpose of the present study was to assess the sensitivity and specificity of the immunofluorescent method used in the Immunology Laboratory, Belfast City Hospital, and to compare results with those obtained with an enzyme-linked immunosorbent assay (ELISA) for  $\alpha$  gliadin antibody, developed in St James's Hospital, Dublin.

## PATIENTS AND METHODS

From June 1982 to July 1985 all patients undergoing jejunal biopsy at the Gastroenterology Unit of the Belfast City Hospital also had a fasting blood sample taken for assay of antibody to gliadin. The biopsy was obtained by Crosby capsule which was placed endoscopically at a distance of 30cm from the pylorus.<sup>9</sup> The study group comprised 102 patients (61 females, 41 males); their age range was 15–89 years, their mean age 46.8 years. Fourteen patients were referred with dermatitis herpetiformis, and four patients who had repeat biopsies during the period were also included in the series giving a total of 106 jejunal specimens with corresponding blood samples. The jejunal biopsies were examined and reported by the Histopathology Department of the Belfast City Hospital. Antibody to gliadin was assayed at the time each blood sample was received in the IgG, IgA and IgM classes using an immunofluorescent method described by Unsworth et al,<sup>3</sup> and antibody in any immunoglobulin class at a titre of 1/10 or greater was considered positive. The sera were also stored at  $-20^{\circ}\text{C}$  and at the end of the study period those available were sent to St James's Hospital, Dublin, for assay of  $\alpha$  gliadin antibody using an enzyme-linked immunosorbent assay (ELISA).<sup>7</sup> Testing was carried out without knowledge of the jejunal biopsy appearance, previous results of antibody testing or diagnosis.

## RESULTS

### *Jejunal biopsy findings*

In accordance with accepted criteria,<sup>10</sup> jejunal biopsies reported as total atrophy, sub-total atrophy or as severe partial atrophy were considered significant. With lesser degrees of atrophy (mild or moderate) they were considered to be of uncertain significance. In the study group 30 patients had total, sub-total or severe partial atrophy, 17 had mild or moderate partial atrophy and 59 had normal histology. Of the 14 patients with dermatitis herpetiformis, five had significant jejunal atrophy, six mild or moderate partial atrophy and three were normal.

### *Incidence of antibodies to gliadin*

Of the 30 patients with significant jejunal histology, 20 (67%) had antibody to gliadin in IgG, IgA or both immunoglobulin classes detected using the immunofluorescent method. Immunoglobulin G class antibody to gliadin was detected in 19/30 (63%) patients whereas only 10/30 (33%) had IgA antibody, and IgM class antibody was not detected in any of the samples. Sera for the ELISA assay of  $\alpha$  gliadin antibody were only available in 28 of these 30 cases and antibody (IgG class) was detected in 18 (64%) (Table I). There were 16 patients whose sera were positive with both assays and eight patients in whom both were negative.

TABLE I

*Titres of IgG and IgA class antibody to gliadin measured by immunofluorescence and the presence of IgG antibody to  $\alpha$  gliadin detected by ELISA in patients with significant jejunal mucosal atrophy*

Patient number	Antibody to gliadin measured by		
	Immunofluorescence IgG	IgA	ELISA IgG
1	160	—	+
2	160	10	+
3	—	20	+
4	—	—	—
5	—	—	—
6	—	—	—
7	40	—	—
8	40	10	+
9	80	10	—
10	10	20	+
11	10	—	+
12	10	—	+
13	40	10	+
14	160	40	NA*
15	20	—	+
16	40	—	+
17	—	—	+
18	20	—	+
19	—	—	—
20	80	—	+
21	10	—	+
22	—	—	+
23	—	—	—
24	—	—	—
25	40	10	+
26	20	10	+
27	—	—	—
28	80	80	NA*
29	—	—	—
30	20	—	+
Number positive	19 (63%)	10 (33%)	18 (64%)

\*NA = Not available.

For the cases with mild or moderate partial atrophy two out of 17 had detectable gliadin antibodies using the immunofluorescent method, and the ELISA test for  $\alpha$  gliadin antibody was positive in two different cases (Table II). The incidence of

positivity in this group of patients, using both methods, was less than in the group with significant histology ( $p < 0.005$  using Chi square analysis).

TABLE II

*Incidence of antibody to gliadin measured by immunofluorescence and ELISA in relation to jejunal histology*

<i>Histology</i>	<i>Number of patients with antibody to gliadin measured by</i>	
	<i>Immunofluorescence</i>	<i>ELISA</i>
Total, sub-total or severe partial atrophy	20/30 (67%)	18/28 (64%)
Mild or moderate partial atrophy	2/17 (12%)	2/17 (12%)
Normal	1/59 ( 2%)	3/59 ( 5%)

For the 59 subjects with normal histology, one was positive using both the immunofluorescent and ELISA methods. A further two cases were positive with the ELISA test alone. The percentages positive with both methods were less than for the group with significant histology ( $p < 0.005$ ).

The sensitivity of each test was expressed as the percentage of cases positive in the significant histology group, hence the immunofluorescent method was 67% and the ELISA method 64% sensitive. The specificity was expressed as the percentage of cases which were negative in the normal histology group. For the immunofluorescent method this was 98.3% compared with 95% for the ELISA test.

## DISCUSSION

This study has demonstrated that there is excellent correlation between the presence of circulating gliadin antibodies and severity of jejunal mucosal atrophy. The ELISA test for antibody to gliadin was similar in detection rate to the currently used immunofluorescent test for gliadin antibodies. It could be argued that storage of sera may in some way have impaired the sensitivity of the ELISA test, but, as a check, the immunofluorescent tests were repeated at the end of the study period on the same stored sera and these showed no difference from the original results.

Although the specificity of both tests was high, their sensitivities were unacceptably low (67%, 64% respectively) with one-third of patients with gluten enteropathy being undetected. Previous studies indicate that detection of gliadin antibodies is more likely amongst children than amongst adult patients with coeliac disease.<sup>1, 6</sup> In our study the age range of patients with significant jejunal mucosal atrophy was 17 to 81 years, mean age 45 years, and therefore our conclusions can only be applied to an adult population.

In a previous study of a group of patients with coeliac disease, in the Republic of Ireland, the detection of  $\alpha$  gliadin antibody by the ELISA technique had a sensitivity of 82% and a specificity of 85%.<sup>7</sup> This higher incidence of both true positives and false positives may reflect genetic differences between the populations in different parts of Ireland.

In the group with mild or moderate villous atrophy, it is probable that some of these cases did not have gluten sensitivity. However, four of the biopsies in this group were repeat tests taken after a gluten-free diet and showed significant histological improvement compared with their initial biopsies. Also a further patient from this group showed significant improvement in a subsequent biopsy after a gluten-free diet. These five patients were therefore definitely gluten-sensitive, but only one had detectable antibody (by immunofluorescence). Although the number of patients was small, this observation tends to emphasise the association of detectable antibody with severe histological changes and not with gluten sensitivity *per se*. This is in keeping with a number of prospective studies which have demonstrated that antibodies to gliadin tend to disappear in patients who take a gluten-free diet and who show histological improvement.<sup>2, 5, 7</sup>

The specificity of both tests was very favourable but there were a small number of false positives in the group with normal histology. One patient who had Crohn's disease had antibody with both assays. Such false positives have been described in Crohn's disease and in various other conditions including ulcerative colitis and postenteritis syndrome.<sup>6</sup>

The presence of antibodies to gliadin in IgA class have been described as being a better indicator of gluten enteropathy than those in IgG class<sup>4, 5, 6</sup> but this was not evident from the present study, when their incidence was lower. In our hands, measurement of IgA antigliadin antibodies alone would be less useful than both IgG and IgA. As guidance to the clinician we have shown that a positive test for gliadin antibody with the currently used immunofluorescent method is highly specific. There is a strong chance that a positive result indicates gluten enteropathy. However, a negative result does not exclude it, and the present sensitivity makes it unsuitable for use as a single screening test.

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